Going after the Real Killer: Metastatic Cancer

Until recently, metastatic disease was considered part of the continuum of cancer progression resulting from accumulated mutations—a late stage of a unified disease process in which primary tumor cells acquire the ability to migrate away from their initiation site to invade and proliferate in different organs. Although it is true that metastases exert their life-threatening effects well after the primary tumor has become a cause for serious concern, recent research indicates that the seeds of metastatic destruction are sown relatively early on. Furthermore, several lines of evidence suggest that metastatic disease operates through molecular mechanisms distinct from those involved in the development of primary tumors.

Within CCR, several principal investigators are converging on the importance of research specifically aimed at stopping cancer metastases. "The emphasis to date in cancer research and in pharmaceutical development has been on trying to treat and eradicate the primary cancer," noted Jeffrey Green, M.D., Head of the Transgenic Oncogenesis and Genomics Section in CCR's Laboratory of Cancer Biology and Genetics. "And the therapeutic strategies for treating primary tumors may not be the same as those needed to treat metastases."

Kent Hunter, Ph.D., Head of the Metastasis Susceptibility Section, which is also in CCR's Laboratory of Cancer Biology and Genetics, agrees. "For breast cancer and many other cancers, we all focus on the primary tumor. That's the wrong thing to focus on because, more often than not, you solve the primary tumor with surgical resection. What kills people is metastasis."

Metastasis Suppressor Genes

More than 20 years ago, as a Postdoctoral Fellow new to NCI, Patricia Steeg, Ph.D. (now Head of the Women's Cancers Section of CCR's Laboratory for Molecular Pharmacology), launched her quest to study the difference between tumor cells that metastasize and those that do not. She decided to study the differences in gene expression between metastasizing and non-metastasizing cell lines derived from the same tumor, hoping to find genes highly expressed in metastatic lines. It was not until she heard a seminar describing the first tumor suppressor gene, *Retinoblastoma* (*Rb*), that she realized the significance of a gene she called *Nm23* (*non-metastatic gene 23*), whose expression was instead reduced in metastatic cell lines. Steeg and her colleagues reintroduced *Nm23* into a highly metastatic melanoma cell line and found that although the cells still made primary tumors when injected into mice, there was a 90 percent reduction in metastases. *Nm23* would be the first identified metastasis suppressor gene.

"Initially, that was an extraordinarily controversial observation," remembered Steeg ruefully. "People looked at metastasis back then and said it was too heterogeneous and unstable to have consistent molecular pathways underlying it." There are now, however,

(Photo: R. Baer)

Patricia Steeg, Ph.D.

(Photo: R. Baer)

Jeffrey Green, M.D.

lung metastases by the end of the study. Kathy Miller, M.D., at the University of Indiana University's Simon Cancer Center is currently leading a Phase II multicenter trial for the use of MPA in the treatment of metastatic breast cancer, a study that stems from Steeg's preclinical work on *Nm23*.

Steeg and her colleagues are also looking for other targets in the *Nm23* pathway that may influence metastasis. To find molecular targets that are suppressed by *Nm23* and potentially involved in promoting metastasis, they have asked which genes are expressed in a pattern that inversely correlates with *Nm23* expression. One promising candidate, EDG2 (*e*ndothelial *d*ifferentiation, lysophosphatidic acid *G*-protein-coupled receptor, 2), appears to be sufficient to restore metastatic growth to cells in which *Nm23* functions

more than 20 known metastasis suppressor genes. These genes are not effective in stopping the growth of primary tumors, but they do stop spreading and/or growth at a distant site. "You have to come to the conclusion that growth of a primary tumor is fundamentally different than the growth of a metastasis."

And where it has been studied, a number of preclinical drug studies have found differential sensitivity of primary and metastatic growth. "We are trying to treat metastatic disease, but we are not developing drugs for it," cautioned Steeg even as she attempts to redress this therapeutic imbalance.

A proportion of breast cancers lose expression of the *Nm23* gene. Steeg and her colleagues showed that high-dose medroxyprogesterone acetate (MPA)—a synthetic progestin hormone used historically in the treatment of endometrial cancers as well as a component of hormone replacement therapy—works atypically through a class of steroid receptors (glucocorticoid receptors) not normally associated with progestin to turn expression of the *Nm23* gene back on. The researchers went on to demonstrate in a mouse model of breast cancer metastasis to the lungs that MPA caused a 60 percent reduction in overt

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as a metastasis suppressor. Steeg's team is currently asking whether EDG2 inhibitors will have anti-metastatic effects in preclinical models.

One Molecule, Two Different Effects on Cancer

Around the same time that Patricia Steeg was embarking on her work with metastasis suppressor genes in the 1980s, Lalage Wakefield, D.Phil., now Head of the Cancer Biology of TGF-β Section in

CCR's Laboratory of Cancer Biology and Genetics, was also beginning her work as a Postdoctoral Fellow at NCI on another molecular player in metastasis. However, it took her a little while to realize where her research was leading.

"TGFs [transforming growth factors] had just been described in the literature," explained Wakefield, an echo of the excitement from those early days still in her voice. TGFs were secreted by cancer cells and were able to transform normal fibroblasts into a premalignant state. "It
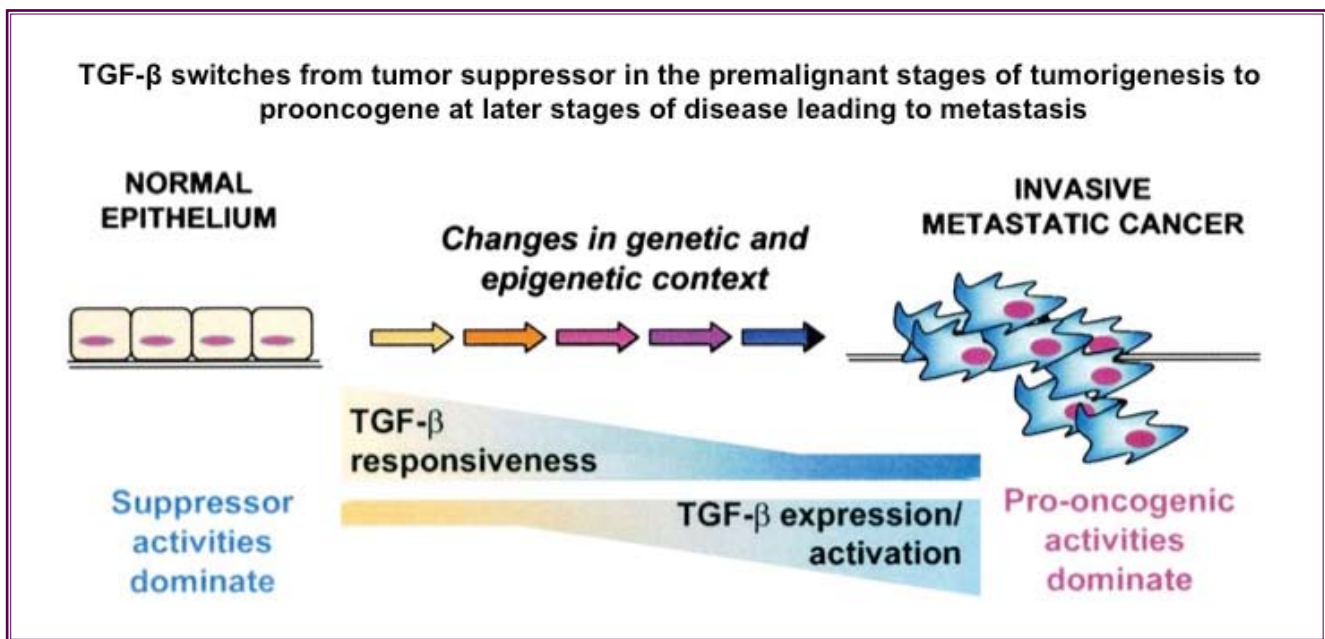
TGF-β switches its role from suppressing tumors before malignancy sets in to promoting metastasis at later stages of disease.

seemed to me that TGFs were going to be the answer to cancer. If we could purify and block these factors, then that would be cancer cured."

TGF-β was eventually discovered to have multiple roles in several different tissues and cell types. It was found to be a key regulator of immune system function, as well as a potent inhibitor of proliferation of normal epithelial cells. Importantly, the TGF-β pathway was genetically inactivated in a number of different cancer types and became known, paradoxically, as a tumor suppressor. Several preclinical studies and mouse models later, the dual role of TGF-β in cancer progression was finally revealed. In the early stages of cancer progression, TGF-β does indeed have tumor suppressor activity, inhibiting proliferation and maintaining genomic stability. As cancer progresses, tumor cells progressively alter their responsiveness to TGF-β. At that stage, TGF-β promotes cell migration, promotes invasion of cancer cells into different tissues, and becomes a pro-survival factor. Meanwhile, TGF-β acts on other cell types, such as fibroblasts, to promote angiogenesis, secrete different

types of molecules into the extracellular matrix, and suppress immune surveillance. In short, TGF-β can promote metastasis through multiple routes.

"TGF-β is a master regulator that sits at the interface of the tumor [and its cellular environment]. It affects every cell that comprises that ecosystem," concluded Wakefield. A molecule with so many diverse effects, operating differently at different stages of cancer progression, would seem to be a pharmaceutical drug developer's nightmare. No one was more surprised than Wakefield and her colleagues, therefore, when they were able to genetically engineer a mouse to encode an inhibitor of TGF-β in its genome and found that this inhibitor protected mice against metastasis in a genetic model of breast cancer. The team has since followed up this work with further preclinical studies that support the use of TGF-β inhibitors to treat metastatic cancer in the clinic. As a result, NCI investigator John Morris, M.D., is now leading a Phase I clinical trial to test GC1008, a human monoclonal antibody against TGF-β in patients with locally advanced or metastatic renal

cell carcinoma or malignant melanoma. The trial is in an extension phase at the highest dose and appears to be showing some promising effects.
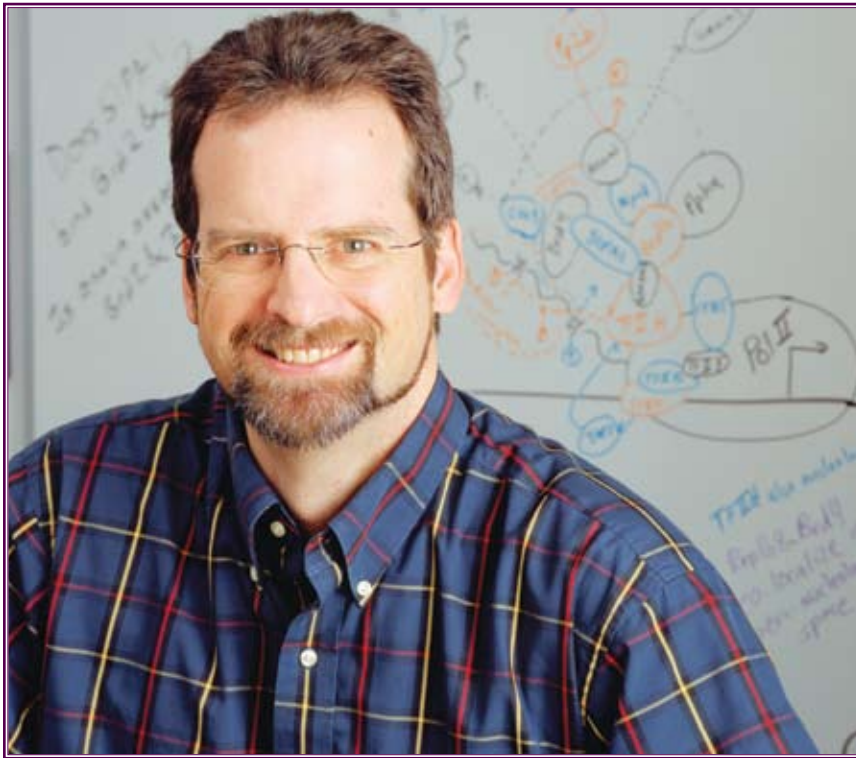
"It's been an incredibly exciting story so far because I have seen this molecule go from its initial discovery and identification to clinical testing, and believe me, it was not a straightforward process," said Wakefield.

Genetic Susceptibility
No one doubts that acquired mutations in individual genes play a critical role in cancer. But, noted Hunter, "You can't look at these things in isolation." He cites the fact that women with BRCA1 mutations do not always develop cancer. "You have to understand the genetic context."

Hunter has taken a population genetics approach to ask whether there are inherited risk factors associated with metastatic progression in cancer. Using a transgene to induce metastatic mammary tumors in several genetically distinct strains of mice, Hunter has shown that the metastatic efficiency, as measured by the density of pulmonary metastases in these mice, varies enormously with

(Photo: R. Baer)

Kent Hunter, Ph.D., looks for inherited risk factors associated with metastatic progression.

The Extracellular Matrix

Hunter and his team have been working to identify the genes that underlie risk of metastatic disease. One focus of their work is the extracellular matrix (ECM), the complex molecular environment that cells both secrete and live in, which provides physical scaffolding through which cells migrate as well as transmit signals to and from cells. Many studies, including Hunter's own work, have shown that changes in the expression of genes encoding ECM molecules predict metastatic progression in both human breast cancer and mouse models. Hunter and his colleagues have begun to identify factors that specifically modulate both ECM-related gene expression as well as metastatic tumor progression. Although we are still far from a mechanistic understanding of how changes in ECM gene expression impact metastasis, the relationship makes some intuitive sense. "I think it has to do with the way cells sense their microenvironment through ECM signaling," said Hunter. For example, the ECM could sequester or modulate the availability of TGF-β and other cytokines involved in growth and immune regulation.

Jeffrey Green and his colleagues have also followed up on the evidence for involvement of the ECM in metastasis. Like Hunter, Green has wondered whether it is not the accumulation of new genetic abnormalities that causes a disseminated but dormant tumor cell to proliferate into clinical disease, "but that something else in the immediate environment or within the host may lead to the trigger that allows these cells to proliferate." Green suspects that there may be critical changes in the composition and structure of the ECM that could allow tumor cells to read different stimulatory signals and initiate a proliferative response.

But dormancy really just means that the disease is subclinical and that doctors cannot see it. How do you find a dormant cell to study it? "Dormancy," Hunter explained, "gives people the idea that it's an inactive seed, a spore sitting somewhere. That's obviously not true—they are cells. We don't know if they are static, or patrolling the body like a lymphocyte."

genetic background. Polymorphisms—DNA sequence differences among individuals—account for variations in many normal physiological traits, such as body size and coloring. Polymorphisms also account for different levels of gene expression, and they account for variation in primary tumors from a variety of tissues. In hindsight, then, it is not surprising that inherited genetic differences would affect the development of metastatic cancer.

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In humans, research has shown that metastatic cells, like primary tumor cells, can be characterized by a gene expression "signature" and that this signature can be used to predict the likelihood of metastasis. Although Hunter does not dispute these findings, and has even found the same genetic signatures in mice with high risk of developing metastasis, he does argue against the interpretation that this signature represents only the accumulation of genetic mutation creating metastatic cells. Instead, he has shown that these signatures can be explained by an interaction of both mutation and genetic background and that non-cancerous tissue from animals with high metastatic risk also has similar gene expression profiles.

But, despite the growing body of evidence that he and his colleagues have developed, skeptics remain. Hunter thinks part of the difficulty is in the scientific culture. "We are trained to think in terms of somatic mutation as cancer biologists. And there's a big divide between susceptibility and somatic genetics in which defects acquired from genetic mutation and rearrangement—not inheritance—are at play.

"We're taught to reduce complexity," concluded Hunter. "But we actually have to embrace it."

Green and Dalit Barkan, Ph.D., a Visiting Scientist, recently reported the development of a three-dimensional culture system model of metastatic cancer that will allow them to address some of the questions of molecular and cellular mechanisms that are so difficult to tackle in this disease. They have shown that cell lines that proliferate in normal cell culture but that can be distinguished by their metastatic potential *in vivo* can also be distinguished in their three-dimensional culture system. Thus, they have been able to study the transition from quiescence to proliferation of metastatic cells, and they have demonstrated a role for the extracellular microenvironment in regulating the reorganization of internal cellular structure that occurs during the switch from dormancy to proliferation. The molecules involved in this reorganization could represent additional targets for metastatic inhibitors (see "Let Sleeping Micrometastases Lie" in Vol.2, No.2 of *CCR connections*).

Finding a Cure

"I am not certain that we will ever be able to completely cure metastatic cancer," said Hunter, realistically and without pessimism. "We should also think about treating it the same way people treat heart disease, by looking for ways to reduce the risk of developing metastatic disease." Hunter's lab has shown that high doses of caffeine suppress metastasis in their mouse model. Although the work does

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not support a recommendation for cancer patients to drink liters of coffee every day, it does indicate that small-molecule agents might be developed for chronic administration to patients that would reduce the risk of metastasis, a strategy

(Image: R.Baer)

Postdoctoral Fellow Daniel Fitzgerald, Ph.D., slices brain tissue for analysis.

analogous to the administration of statins to reduce the risk of heart disease.

Wakefield's work with TGF-β, which has effects on so many different physiological systems, has led her to the conclusion that combinations of drugs with different molecular targets will be an important part of the solution. Her work suggests that a combination of a lot of small effects on different cell types involved in the metastatic process would be most effective in combating the disease.

"The major stumbling block," Steeg pointed out, "is how to test our preclinical data in the clinic. Most of our data says that if we use drug X, we can prevent metastasis, but standard clinical trials start with a Phase I trial in highly metastatic patients—so you are asking a drug to melt a golf ball-sized tumor. Most agents will fail in that trial design [even though they might be effective

when administered earlier]." Steeg suggests that including biopsies that demonstrate whether the drug had an effect on its target may be a first step. Better imaging tools will also be critical. But, ultimately, we may need to rethink how we do clinical trials.

Steeg has recently formed a Center of Excellence to study brain metastases of breast cancer, a disease that combines all of the difficulties in studying metastatic disease with the need to find drugs that cross the blood-brain barrier that normally protects the brain from most blood-borne molecules. The current standard of care, whole brain radiation therapy, may be successful in eradicating the tumors for a time, but it may have serious neurological side effects. The Center's work, which has been funded by a five-year grant of over $17,000,000 from the Department of Defense Breast Cancer Research

Lalage Wakefield, D.Phil.

Program, is a comprehensive program ranging from target identification to drug delivery methods. The researchers that form this center include neuropathologists, neurosurgeons, neuro-oncologists, molecular biologists specializing in breast cancer, and experts on the blood-brain barrier. "We each have our assignment—we need more model systems, and we need more tissue studies," Steeg concluded (see also "Small Molecule, Big Impact" in Vol.2, No.2 of *CCR connections*).
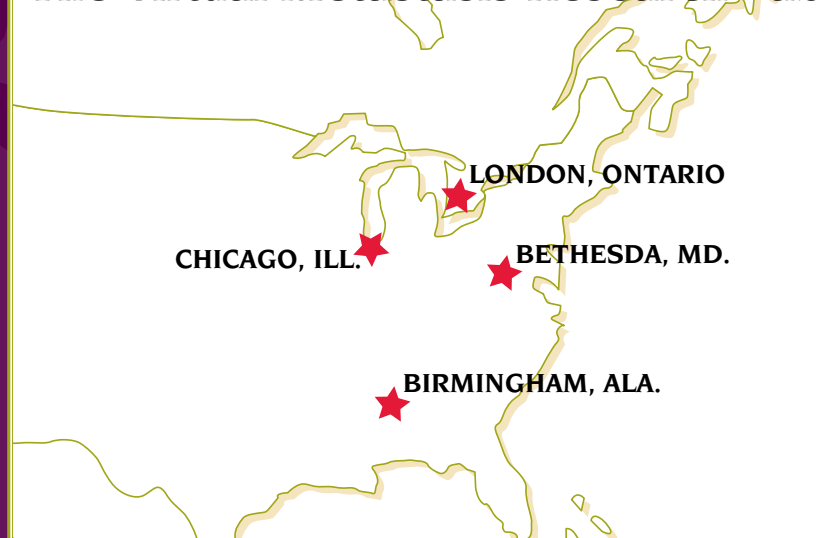
To learn more about Dr. Hunter's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?Name=hunter.

To learn more about Dr. Green's research, please visit his CCR Web site at http://ccr.cancer.gov/staff/staff.asp?Name=green.

To learn more about Dr. Steeg's research, please visit her CCR Web site at http://ccr.cancer.gov/staff/staff.asp?Name=steeg.

To learn more about Dr. Wakefield's research, please visit her CCR Web site at http://ccr.cancer.gov/staff/staff.asp?Name=wakefield.

The Virtual Metastasis Research Lab

LONDON, ONTARIO

CHICAGO, ILL.

BETHESDA, MD.

BIRMINGHAM, ALA.

The Virtual Metastasis Research Lab (VMRL) comprises laboratories from several cities across North America.

Virtual Metastasis Research Lab

Every month, a group of cancer researchers gets together to discuss the latest results of their work in an informal setting. They discuss unpublished results, solicit each other's help in understanding their data, and toss around a few wild ideas. This situation sounds like a typical lab meeting, except that the researchers come from many different laboratories, both within CCR and at universities across the country, and they meet online using Web-based conferencing tools.

Kent Hunter, Ph.D., organized the Virtual Metastasis Research Lab (VMRL), which evolved from a CCR working group on metastasis. "Metastasis is an organismal disease," said Hunter, noting that solving it will require researchers with a diverse set of expertise. "Lots of different views on the same data open up interesting ideas for people to try. Everyone contributes in different ways."

Lalage Wakefield, D.Phil., agrees. "It's very interactive, there's a lot of discussion, and we really benefit from having great people on the outside as well as the ones we have here." Wakefield also likes the way it has helped to encourage collaboration within CCR. She points to the development of a lung slice culture system to study the early events of metastatic cell seeding that started as a casual conversation between her and Hunter about the need for an intermediate system between purely *in vitro* approaches and animal models. They took their notion to their colleague Chand Khanna, D.V.M., Ph.D., Head of the Tumor and Metastasis Biology Section in CCR's Pediatric Oncology Branch, who turned around and created it.

The VMRL has entered its third year, and it includes approximately 50 people from the participating laboratories. "I think it's helped bring people within NCI as well as the extramural participants much closer together," said Jeffrey Green, M.D. "Instead of seeing them at a meeting once a year, we talk to each other all the time."